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Alpha/Beta Interferons Increase Host Resistance to Murine AIDS

JASMINE K. M. HENG, PATRICIA PRICE, C. MAY LAI, AND MANFRED W. BEILHARZ*

Department of Microbiology, Queen Elizabeth II Medical Centre, University of Western Australia, Nedlands 6009, Western Australia, Australia

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Murine AIDS (MAIDS) is caused by a defective retrovirus present in the LP-BM5 murine leukemia virus mixture. Strains of inbred mice differ in resistance to MAIDS development; some are susceptible (e.g., C57BL/6), while others are resistant (e.g., CBA and B10.BR). As an early block to viral replication in resistant mice has been demonstrated previously by PCR studies, we postulated that alpha/beta interferons (IFN-α/β) may be involved in resistance to MAIDS. Susceptible C57BL/6 mice infected with LP-BM5 were treated with IFN-α/β or Newcastle disease virus. Newcastle disease virus induces high endogenous IFN-α/β production in mice. Both treatments delayed the development of MAIDS, as assessed by splenomegaly and T- and B-cell proliferation. In addition, an IFN-α/β response was detected by reverse transcription-PCR and dot blotting 3, 6, and 9 h after LP-BM5 infection in resistant mice but not in susceptible mice. These results suggest that the ability to produce IFN-α/β in response to LP-BM5 infection may contribute to host resistance to MAIDS.

Retrovirus-induced murine AIDS (MAIDS) is caused by LP-BM5 murine leukemia virus (MuLV) and exhibits many similarities to the human AIDS-related complex (3). LP-BM5 MuLV is a mixture of retroviruses consisting of a nonpathogenic, replication-competent, B-cell-tropic, ecotropic virus (BM5e); a mink cell focus-inducing virus (BM5-MCF); and a replication-defective etiologic virus (BM5d). LP-BM5 MuLV induces a progressive lymphoproliferative disease (MAIDS) characterized by polyclonal T- and B-cell activation, enhanced production of several cytokines, hypergammaglobulinemia, and immunosuppression (20). The mechanism by which BM5d induces MAIDS is still unknown, but functional B and T cells are essential for the development of the disease (5, 21). A small defined region of the defective virus genome has been found to be essential for the pathology to develop (15, 24).

Different strains of mice show various susceptibilities to MAIDS development. Susceptible mice (e.g., C57BL/6) develop MAIDS in 3 to 10 weeks after infection with LP-BM5 MuLV, whereas resistant mice (e.g., CBA/CaH) may remain symptom free. In addition, some mouse strains (e.g., BALB/c) show intermediate susceptibility to MAIDS (13, 19). The *H-2*^{b} genotype and non-major histocompatibility complex-linked loci affect the development of MAIDS (12, 18) but the genetic basis for resistance has not been established. It has been shown by PCR techniques that resistant mice show a very early block to replication of both BM5d and BM5e, while susceptible mice do not (25). This very early block in viral replication was suggestive of an alpha/beta interferon (IFN-α/β) effect. The present study examined the role of IFN-α/β in MAIDS progression.

IFNs are soluble proteins which possess antiviral, antiproliferative, and immunoregulatory properties. The type 1 IFNs are IFN-α, IFN-β, and IFN-ω, whereas IFN-γ is a type 2 IFN (23). IFN-α is induced by human immunodeficiency virus type 1 (HIV-1) infection of peripheral blood mononuclear cells (2) and inhibits the early stages of the HIV-1 replication cycle specifically at the level of provirus formation (28, 29). IFN-β enhances resistance of peripheral blood leukocytes to HIV-1 infection (32). IFN-α and IFN-β decrease HIV-1 infection of human umbilical vein endothelial cells (26), and a *tat*-activated, transduced IFN gene can inhibit HIV-1 replication in T cells and monocytes (30).

In this study, murine IFN-α/β (MuIFN-α/β) was either administered to mice 2 h after LP-BM5 by direct intraperitoneal (i.p.) injection or induced in vivo by Newcastle disease virus (NDV) infection 3 h prior to LP-BM5 infection. The progression of MAIDS was then monitored for 12 weeks. In addition, spleens and livers from MAIDS-susceptible and -resistant mice were screened for IFN-α/β in the first few hours after LP-BM5 infection. We found that IFN-α/β significantly slowed the development of MAIDS in susceptible mice (C57BL/6 and B6.C-*H-28*^{c}) and that resistant mice (CBA and B10.BR) exhibited a stronger IFN-α/β mRNA response to LP-BM5 infection than did susceptible mice. The results suggest a role for IFN-α/β in resistance to the disease.

MATERIALS AND METHODS

Mice. Six-week-old female C57BL/6, CBA.CaH, B10.BR, BALB.B, and B6.C-*H-28*^{c} mice were obtained from the Animal Resource Centre (Murdoch University, Murdoch, Western Australia, Australia) with specific-pathogen-free status. During the experiments, mice were housed in a temperature- and humidity-controlled holding center under strict barrier conditions with a 12-h light–12-h dark cycle. All procedures were approved by the Animal Welfare Committee of the University of Western Australia.

Virus stocks. LP-BM5 MuLV was administered as a supernatant from SC1-G6 cells chronically infected with BM5e, BM5d, and BM5-MCF viruses (6) and titrated in C57BL/6 mice such that splenomegaly developed 6 to 8 weeks postinfection (p.i.). NDV was prepared by inoculating an NDV isolate into the allantoic fluid of 9- to 11-day-old embryonated chicken eggs. The allantoic fluid was harvested and clarified 4 days p.i., and NDV titers were assayed as 50% egg-infective doses (17). Encephalomyocarditis virus was propagated in L929 cells for the IFN bioassay (17).

IFN-α/β treatment in vivo. Control mice were administered 100 μl of Dulbecco's modified Eagle medium (DMEM), 100 μl of LP-BM5, or 100 μl of DMEM containing 10,000 IU of MuIFN-α/β (Lee Biomolecular, San Diego, Calif.) i.p. or 100 μl of NDV (10^8 50% egg-infective doses) intravenously. Treatment groups were given either MuIFN-α/β i.p. 2 h after LP-BM5 infection or NDV intravenously 3 h prior to LP-BM5 infection. These regimens were adopted to achieve peak levels of IFN in the serum at the time of LP-BM5 infection. In later experiments, NDV was administered in multiple doses 3 h after LP-BM5 infection and once a week for 12 weeks. Groups of at least five mice were analyzed 4, 8, and 12 weeks p.i. In addition, to determine the kinetics of IFN

* Corresponding author. Phone: 61 (9) 346 2217. Fax: 61 (9) 346 2912. Electronic mail address: beilharz@uniwa.uwa.edu.au.

induction by NDV infection of mice, sera were bioassayed for IFN-α/β 3, 6, and 9 h p.i.

Lymphoproliferation assays. Single spleen cell suspensions were prepared as pools from each group of mice and treated with Tris-NH_4Cl to remove erythrocytes. Cell suspensions (200 μl) were adjusted to 2.5×10^6 cells per ml in *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-buffered RPMI 1640 with 5×10^{-4} M 2-mercaptoethanol and 2% fetal bovine serum. These were stimulated with concanavalin A (0.5 μg per well; Pharmacia, Uppsala, Sweden) or lipopolysaccharide (LPS) (50 μg per well; Sigma) for 24 h. [^{3}H]thymidine (0.5 μCi per well; Amersham) was added, and incorporation was examined 18 h later by liquid scintillation counting.

Induction of IFN-α/β by LP-BM5 in vitro. Spleen cell suspensions were prepared from uninfected mice. Aliquots of 2×10^7 cells were incubated with 1 ml of an LP-BM5 stock or DMEM for 1 h at 37°C. The incubation medium was then removed, and 10 ml of DMEM with 10% fetal bovine serum was added. Supernatants were sampled 3, 6, and 9 h later and screened for IFN-α/β by bioassay.

Direct detection of IFN-α/β produced in vivo. Groups of three to five mice were injected i.p. with 100 μl of DMEM or LP-BM5 and sacrificed after 3, 6, and 9 h. Pooled spleen and liver samples were collected. Half of each organ was frozen in liquid nitrogen for RNA extraction. The other half was weighed and homogenized in mouse osmolarity buffered saline (pH 7.2) for detection of IFN-α/β by bioassay.

IFN bioassay. Homogenates of liver (50% [wt/vol]) and spleen (20% [wt/vol]) tissues, serum samples, and tissue culture supernatants were treated (pH 2.0) to remove acid-labile IFN-γ and titrated in a bioassay based on the inhibition of cytopathic effects in L929 cells infected with encephalomyocarditis virus (14). MuIFN-α/β (Lee Biomolecular) was included as the standard in this assay.

Reverse transcription (RT)-PCR–dot blot hybridization detection of IFN. Total RNA was extracted from spleen and liver samples with RNAzol B (BIO TECX Lab. Inc.) in accordance with the manufacturer's recommendations and treated with DNase (16). The concentration and integrity of the resulting RNA were checked by spectrophotometry and formaldehyde-agarose gel electrophoresis. The RNA (1 μg) was reverse transcribed with 15 U of reverse transcriptase, 25 U of RNasin, 0.5 μg of random hexamers as primers, and a 1 mM concentration of nucleotides in a total volume of 25 μl. The reverse-transcribed material was checked by amplifying β-actin cDNA with a pair of mouse β-actin-specific primers as described previously (22). The PCR resulted in the amplification of a 348-bp fragment. Plasmid pMCQ, which included the amplified actin region, was used as a positive control for the mouse β-actin PCR. A 1.5-μl volume of the cDNA reaction mixture was amplified by PCR to detect MuIFN-α/β with two sets of specific primers. The 25-μl PCR contained 2 U of *Taq* DNA polymerase, 2 mM $MgCl_2$, 0.2 mM nucleotides, and 100 ng of each primer. The presence of *MuIFNA* was indicated by the generation of a 308-bp fragment. The primers used to generate this fragment were 5′-TCTCTCCTGCCTGAAG GAC-3′ and 5′-ACACAGTGATCCTGTGGAA-3′, which correspond to the regions coding for amino acids 26 to 32 and 124 to 130 of MuIFN-α, respectively. These two regions show total sequence conservation for all known *MuIFNA*s. The presence of *MuIFNB* was indicated by the generation of a 390-bp fragment. The primers used for *MuIFNB* detection were 5′-CAGCTCCAAGAAAGGAC GAA-3′ and 5′-GTAGCTGTTGTACTTCATGAG-3′, which correspond to the regions coding for amino acids 7 to 13 and 130 to 136 of MuIFN-β, respectively. The parameters used to amplify IFN-α/β were denaturation at 95°C for 5 min, annealing at 55°C for 2 min, and extension at 70°C for 4 min for the first cycle, followed by denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 70°C for 2 min for another 34 cycles. A 10-μl volume of the RT-PCR products was analyzed on a 2% agarose gel. For increased sensitivity, 1 μl of the RT-PCR products was also dot blotted onto a nylon membrane and hybridized at 65°C to a mixture of [γ-^{32}P]ATP-labelled oligonucleotides specific for *MuIFNA*s (16) and *MuIFNB*. The *MuIFNB*-specific oligonucleotide has the sequence 5′-CGTCTC CTGGATGAACTCCACC-3′, which corresponds to the region coding for amino acids 82 to 89 of the MuIFN-β sequence. A control blot containing amplified *MuIFNA1*, *-A4*, *-A5*, *-A6*, *-A9*, and *-B* DNA clones in 10-fold dilutions ranging from 50 to 0.05 ng was cohybridized with the test membrane.

Statistical analysis. Spleen weights were compared by the unpaired Student *t* test.

RESULTS

IFN-α/β slows the development of MAIDS in susceptible strains. Groups of C57BL/6 and B6.C-*H-28*^{c} mice were infected with LP-BM5 and monitored at 4, 8, and 12 weeks p.i. Splenomegaly and immunodeficiency are characteristic of MAIDS; therefore, the increase in spleen weight and depression of lymphoproliferative responses were used as indices of disease progression. The average spleen weight of uninfected mice was 0.08 ± 0.02 g (Fig. 1a and b and 2 and Table 1). There was no apparent increase in average spleen weight in resistant mouse strains (CBA.CaH and B10.BR) up to 12 weeks p.i. (data not shown). In contrast, there was a marked increase in

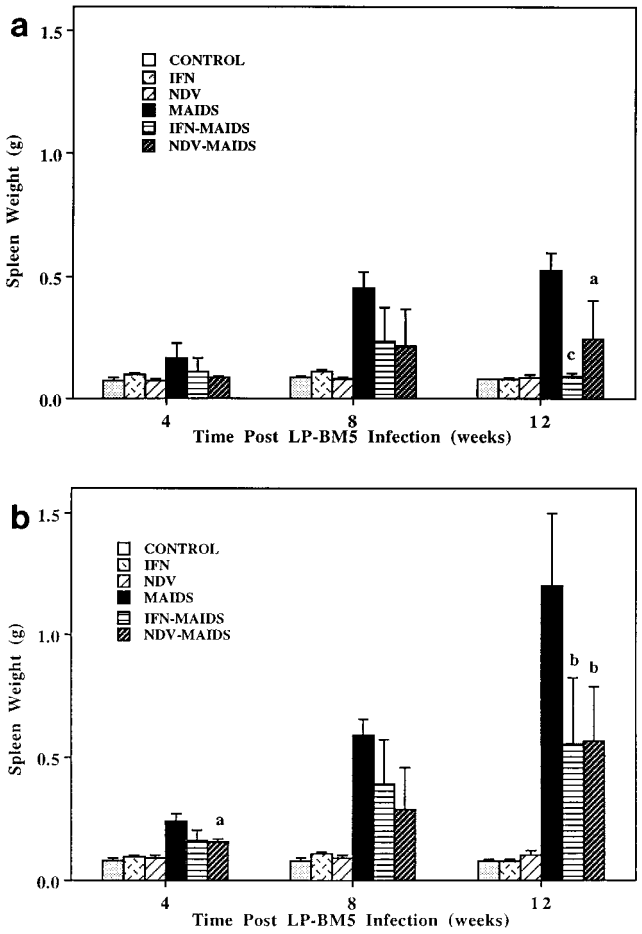

FIG. 1. Effects of IFN-α/β and NDV infection on spleen weights of B6.C-*H-28*^{c} (a) and C57BL/6 (b) mice. Groups of five mice were sampled 4, 8, and 12 weeks p.i. with LP-BM5, and results are presented as means ± the standard deviations. Experiments were conducted twice. CONTROL, no infection or treatment; IFN, IFN treatment only; NDV, NDV infection only; MAIDS, LP-BM5 infection only; IFN-MAIDS, IFN treatment 2 h after LP-BM5 infection; NDV-MAIDS, NDV infection 3 h prior to LP-BM5 infection. There were significant differences between groups marked with letters and mice given LP-BM5 only (Student *t* test): a, $P < 0.05$; b, $P < 0.005$; c, $P < 0.0005$.

average spleen weight in susceptible B6.C-*H-28*^{c} (Fig. 1a) and C57BL/6 mice (Fig. 1b and 2). The average spleen weights of LP-BM5-infected C57BL/6 mice were also generally higher than those of LP-BM5-infected B6.C-*H-28*^{c} mice.

IFN-α/β was administered i.p. at a dose of 10,000 IU 2 h after LP-BM5 infection. The single-dose MuIFN-α/β treatment was able to reduce spleen weight increases in LP-BM5-infected B6.C-*H-28*^{c} (Fig. 1a; $P < 0.0005$ at 12 weeks p.i.) and C57BL/6 (Fig. 1b; $P < 0.05$ and $P < 0.005$ at 4 and 12 weeks p.i., respectively) mice compared with mice infected with LP-BM5 only. A way of inducing IFN-α/β in mice is to infect them with NDV, which is nonpathogenic in mice (7). The kinetics of IFN-α/β induction by NDV infection of C57BL/6 and B6.C-*H-28*^{c} mice are shown in Fig. 3. NDV infection of both strains induced high levels of IFN-α/β in serum, which were maximal at 3 h p.i. (Fig. 3). Therefore, mice were pretreated with NDV 3 h prior to LP-BM5 infection to ensure high levels of IFN-α/β circulating in the serum of the mice at the time of LP-BM5 infection. Spleen weights were again significantly lower in NDV-pretreated LP-BM5-infected mice than in mice given

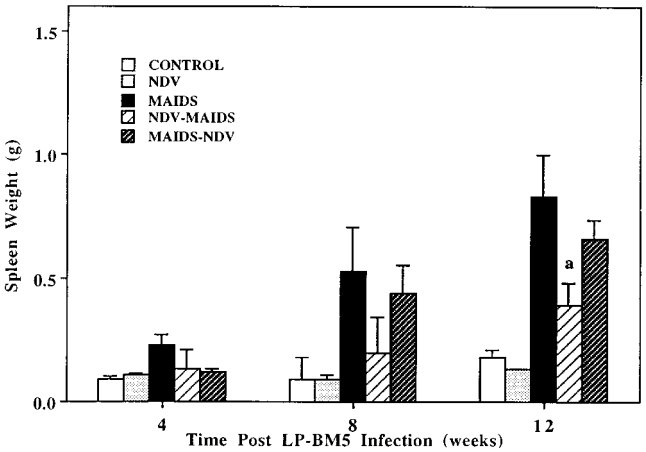

FIG. 2. Effects of NDV infection on spleen weight in C57BL/6 mice. Groups of three to five mice were sampled 4, 8, and 12 weeks p.i. with LP-BM5, and results are presented as means ± the standard deviations. Experiments were conducted twice. CONTROL, no infection or treatment; NDV, NDV infection only; MAIDS, LP-BM5 infection only; NDV-MAIDS, NDV infection 3 h prior to LP-BM5 infection; MAIDS-NDV, NDV infection 3 h after LP-BM5 infection and once a week thereafter for 12 weeks. There was a significant difference between the group marked with the letter a and mice given LP-BM5 only (Student *t* test; $P < 0.05$).

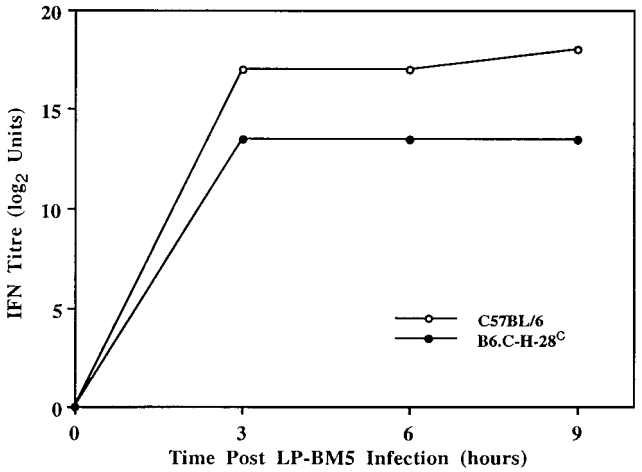

FIG. 3. Induction of IFN-α/β in serum of C57BL/6 (○) and B6.C-*H-28*^{c} (●) mice 3, 6, and 9 h after NDV infection. IFN titers were measured by bioassay and are presented as log_2 units per milliliter.

only LP-BM5. This was evident in MAIDS-susceptible B6.C-*H-28*^{c} mice (Fig. 1a; $P < 0.05$ at 12 weeks p.i.) and C57BL/6 mice (Fig. 1b; $P < 0.005$ at 12 weeks p.i.). Interestingly, NDV treatment 3 h after LP-BM5 infection and multiple (weekly) doses of NDV did not slow MAIDS progression as effectively as did the single early dose of NDV (Fig. 2).

The incorporation of [^{3}H]thymidine into stimulated T and B cells in spleen preparations from LP-BM5-infected mice is known to be depressed by MAIDS. Results obtained with B6.C-*H-28*^{c} spleen cells collected 8 weeks after LP-BM5 infection are shown in Table 1. The depression of responses to concanavalin A and lipopolysaccharide parallelled the extent of splenomegaly. Proliferative responses of the LP-BM5-infected mice treated with NDV or IFN were lower than those of uninfected mice but higher than those of mice infected with only LP-BM5. Similar results were obtained with C57BL/6 mice (data not shown).

Direct detection of IFN-α/β in MAIDS in vivo. As MuIFN-α/β administration or NDV induction of MuIFN-α/β at the time of LP-BM5 infection slowed the progression of MAIDS, we hypothesized that a difference in the kinetics or magnitude of the IFN-α/β response to LP-BM5 may help suppress the development of disease in some or all strains of resistant mice. IFN-α/β was not detected by bioassay of culture media from splenocytes infected with LP-BM5 in vitro. Spleen and liver homogenates collected 3, 6, and 9 h after infection with LP-BM5 in vivo also showed no IFN-α/β bioactivity. However, RT-PCR is a very sensitive method of detection of MuIFN-α/β mRNA and provided evidence of a differential response. Intact total RNA (Fig. 4A) was reverse transcribed, equal amounts of cDNA (confirmed by β-actin PCR, Fig. 4B) were amplified with primers specific for IFN-α/β sequences, and this revealed that *MuIFNA* mRNA (308 bp; lower band) was present at 3 h p.i. in liver samples from infected MAIDS-resistant CBA mice (Fig. 4C, lane 1). Similar faint bands were visible at 6 and 9 h p.i. in the livers and at 3, 6, and 9 h p.i. in the spleens of CBA mice (data not shown). In contrast, IFN-α/β was not detected by RT-PCR amplification of spleen or liver samples from the MAIDS-susceptible C57BL/6 strain at 3, 6, and 9 h p.i. Results obtained with liver samples collected at 3 h p.i. from C57BL/6 mice are included in Fig. 4 (lane 3). Although ethidium bromide staining of the PCR products on agarose gels showed a low-level *IFNA* response in resistant mice, the IFN signal was weak (Fig. 4C, lane 1). Dot blot hybridizations (*MuIFNA1*, *-A4*, *-A5*, *-A6*, *-A9*, and *-B*) of the PCR products to a mixture of *IFNA*- and *IFNB*-specific probes were used to enhance the signal observed. IFN signals were detected in liver and spleen samples collected from resistant mice at 3 and 6 h p.i. (Fig. 5A), and signals from infected mice were much stronger than those from uninfected control mice. No signals were detected

TABLE 1. Proliferative responses of splenocytes prepared from B6.C-*H-28*^{c} mice 8 weeks after LP-BM5 infection[a]

Treatment group[b]	Mean spleen wt (g) ± SD	Mean [^{3}H]thymidine incorporation (dpm, 10^3) ± SD	
		Concanavalin A	Lipopolysaccharide
Control	0.082 ± 0.007	190.5 ± 57.1	82.3 ± 1.8
MAIDS	0.450 ± 0.067	2.9 ± 1.9	11.3 ± 1.7
NDV	0.078 ± 0.007	142.5 ± 38.8	76.0 ± 1.9
NDV-MAIDS	0.212 ± 0.150	108.5 ± 6.6	44.6 ± 0.9
IFN-MAIDS	0.176 ± 0.167	80.3 ± 4.2	34.9 ± 3.5

[a] Experiments were conducted twice.
[b] Control, no infection or treatment; MAIDS, LP-BM5 infection only; NDV, NDV infection only; NDV-MAIDS, NDV infection 3 h prior to LP-BM5 infection; IFN-MAIDS, IFN treatment 2 h after LP-BM5 infection.

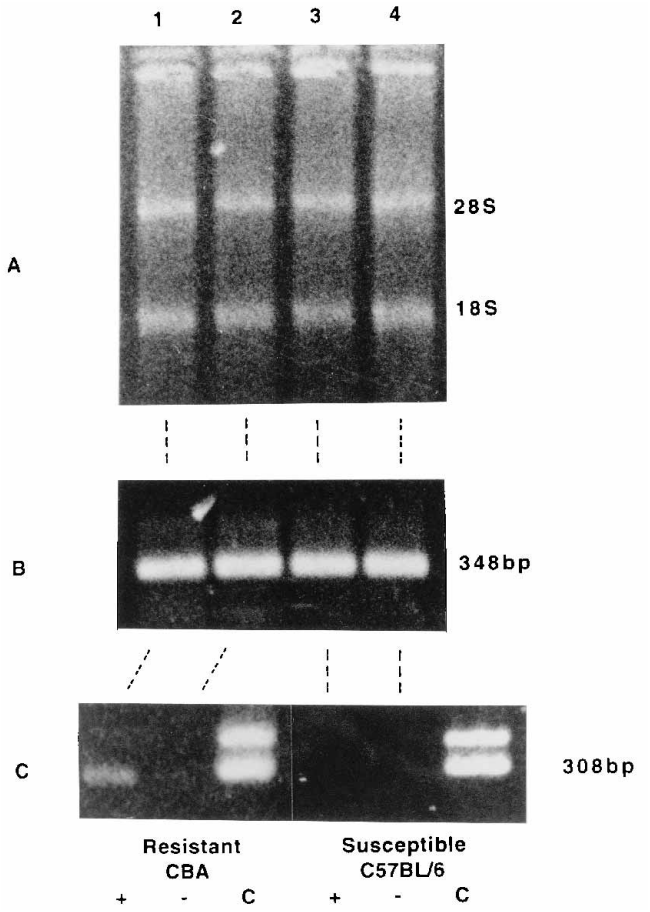

FIG. 4. RT-PCR amplification of IFN-α/β mRNAs from the livers of MAIDS-resistant CBA mice and MAIDS-susceptible C57BL/6 mice 3 h p.i. with LP-BM5. Lanes 1 and 3 contained MAIDS-infected liver samples taken at 3 h p.i., and uninfected control liver samples taken at 3 h p.i. were in lanes 2 and 4. Lanes 1 and 2 contained CBA samples while lanes 3 and 4 contained C57BL/6 samples. (A) Intact 18S and 28S rRNAs present in RNA preparations from livers of infected and control CBA and C57BL/6 mice. (B) Ethidium bromide-stained agarose gel containing mouse β-actin PCR amplification products (348 bp) to confirm that equal amounts of input RNA were present. (C) Ethidium bromide-stained agarose gel containing IFN-α/β bands amplified by PCR. Plus and minus signs, LP-BM5-infected and uninfected control samples, respectively; C, control IFN DNA used in PCR: the upper band is *IFNB* (390 bp), and the lower band is *IFNA* (308 bp).

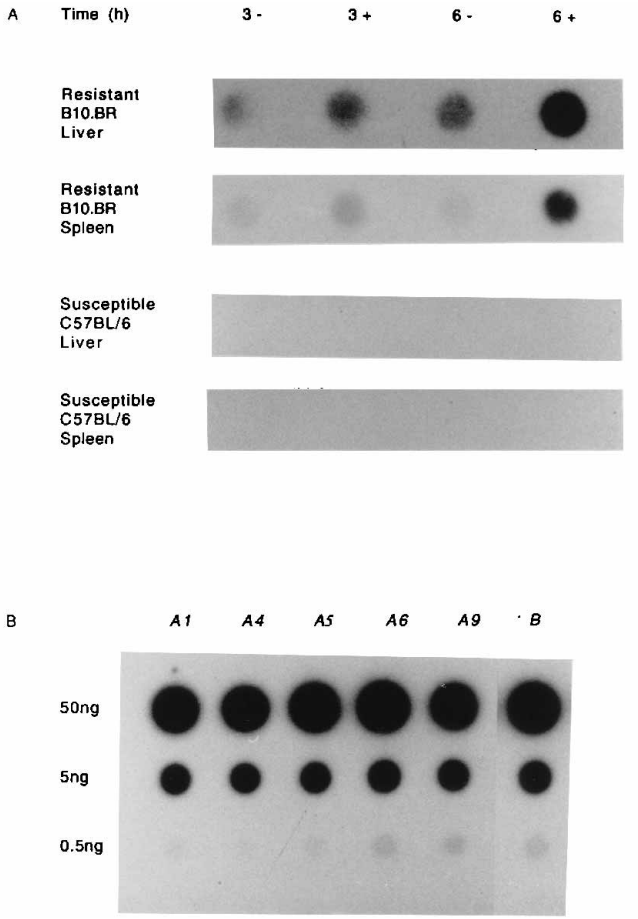

FIG. 5. Dot blot hybridization of IFN RT-PCR products from liver and spleen samples of MAIDS-resistant B10.BR mice and MAIDS-susceptible C57BL/6 mice at 3 and 6 h p.i. with LP-BM5. (A) Samples (1.5 μl) of RT-PCR products were immobilized on nylon membranes and probed with a mixture of *IFNA*- and *IFNB*-specific, labelled oligonucleotides including *MulFNA1*, *-A4*, *-A5*, *-A6*, *-A9*, and *-B*. Minus and plus signs, control and infected samples, respectively. (B) Control blot containing amplified *MulFNA1*, *-A4*, *-A5*, *-A6*, *-A9*, and *B* DNA clones, in 10-fold dilutions ranging from 50 to 0.05 ng, cohybridized with the test membrane.

in liver or spleen samples from MAIDS-susceptible C57BL/6 mice. Control blotting (performed simultaneously in the same hybridization bag) showed the specificity of the process (Fig. 5B) in that all six of the IFN-α/β subtypes blotted gave strong signals (see also references 16 and 17 for further details of dot blot specificity).

DISCUSSION

IFN-α/β administered directly to MAIDS-susceptible mice by the i.p. route 2 h after LP-BM5 infection delayed the development of MAIDS. A similar result was obtained by NDV treatment, which induces IFN-α/β in mice. This suggests that IFN-α/βs may have a role in resistance to MAIDS. Interestingly, the time of NDV treatment (or IFN-α/β administration) appeared to be important. Administration of NDV after LP-BM5 infection and in multiple doses provided less protection than NDV treatment 3 h prior to LP-BM5 infection (Fig. 2). Hence, there may be a critical period of time early in MAIDS infection when an IFN-α/β response is required to effect resistance mechanisms.

NDV infection induces IFN-α/β titers in the serum of mice, the amount of which is controlled by the *If-1* locus (7). As evidenced in Fig. 3, C57BL/6 mice express the high-IFN-α/β-producing allele ($If\text{-}1^h$) and produce more IFN-α/β than do B6.C-$H\text{-}28^c$ mice, which express the low-IFN-α/β-producing allele ($If\text{-}1^l$) (28). In the present study, this difference in serum titers was around 4 $\log_2$ U or 16-fold, which is in good agreement with previous studies (28). Our study has shown that NDV infection 3 h prior to LP-BM5 infection significantly impairs the development of MAIDS in both C57BL/6 and B6.C-$H\text{-}28^c$ mice. This finding was contrary to expectations based on the large differences in serum IFN responses. However, the lower levels of IFN produced by B6.C-$H\text{-}28^c$ mice appear to be sufficient or induced rapidly enough to limit MAIDS. Indeed, it is noteworthy that spleen weights of MAIDS-infected B6.C-$H\text{-}28^c$ mice were generally lower than those of C57BL/6 mice (12-week data of Fig. 1a; cf. 12-week data of Fig. 1b and 2). It appears that the decreased susceptibility of the congenic B6.C-$H\text{-}28^c$ strain may be determined by

genes adjacent to the *If-1* locus, which include the histocompatibility locus *H-28*. The *If-1* locus was backcrossed onto the C57BL/6 background from a BALB/c mouse, which has intermediate MAIDS resistance. The implications of this observation are being explored in ongoing experiments.

We have shown that MAIDS progression can be slowed by the presence of IFN-α/β very early in the infection. If IFN is important in host resistance to MAIDS, it should be induced by LP-BM5 infection. Moreover, one may expect higher levels of IFN or higher sensitivity to endogenous IFN to be a feature of one or more strains of mice resistant to the disease. Unfortunately, at 3, 6, and 9 h p.i., we were unable to detect the in vitro or in vivo production of bioactive IFN by spleen cells of resistant mice that had been stimulated with LP-BM5. However, some IFN mRNA was detected in vivo by RT-PCR–dot blot hybridization in resistant mice but was lacking in susceptible mice. IFN mRNA was detected in the liver as early as 3 h p.i. This suggests that there may be no need for virus integration and expression. Rapid responses to NDV (7) or murine cytomegalovirus (1) are also seen in the serum and livers of mice, and these appear to be responses prior to viral gene expression. The failure to detect IFN by bioassay may reflect localized cellular production of IFN which is too low to be detected in whole-organ homogenates. Alternatively, samples may have contained only IFN mRNA which had not been translated into protein at the time of sampling and bioassay (i.e., there may be posttranscriptional control of IFN-α/β expression). Detection of IFN-α/β mRNA by RT-PCR in the absence of detectable bioactivity of the protein has been reported previously (11). IFNs present at levels below the detection limits of the bioassay may act in a paracrine manner in critical target organs, being rapidly taken up by nearby cells such that whole-organ homogenates contain little bioactive material. In the present study, only samples from MAIDS-resistant mice showed *IFNA* mRNA to be present at very early times after MAIDS infection. The ability of these resistant mice to mount such an IFN response appears to contribute to their resistance to MAIDS. It is noteworthy that some *IFNA* mRNA was present in both liver and spleen samples from uninfected, resistant mice. This suggests that these mice have a highly sensitive IFN response that is reminiscent of the constitutive, low-level IFN production seen in some human cells (11). Such low constitutive levels may also act to prime a stronger response to viral stimulation.

During the first week of LP-BM5 infection, there is transient synthesis of interleukin 1 (IL-1), IL-2, and IFN-γ in spleen cells. In the later stages of the infection, spontaneous increases in the expression of IL-4, IL-5, IL-6, and IL-10 were observed (10). The development of MAIDS may be associated with a change in balance between the Th1 (IL-2 and IFN-γ) and Th2 (IL-4, IL-6, and IL-10) phenotypes (4). A recent report (9) has shown that treatment with IL-12, which induces IFN-γ (27), slows the development of MAIDS in C57BL/6 mice. The effects of IL-12 were similar to those of IFN-α/β in the present study. Hence, it is possible that resistance to MAIDS is due, in part, to activation of this IL-12–IFN-γ pathway by the IFN-α/β response in resistant mice, leading to a Th1 state. Further supportive evidence for the IFN-α–IL-12–IFN-γ pathway was presented in a recent report that treatment with a prostaglandin E_2 inhibitor enhanced the synthesis of IFN and reduced the symptoms of MAIDS in susceptible mice (31). However, one group has reported that an IFN-γ-dependent pathway may be involved in the pathogenesis of MAIDS. Our data and the data from the IL-12 (9) and prostaglandin E_2 inhibitor (31) studies appear to support the notion that IFN-γ limits MAIDS development.

In conclusion, this study has shown that IFN-α/β inhibits the progression of MAIDS in susceptible mice. In addition, only MAIDS-resistant mice produce a small but detectable amount of IFN-α/β mRNA in response to LP-BM5 infection. The IFN-α/β response of a mouse strain may therefore have a role in its resistance to MAIDS, possibly via the induction of IL-12, which, in turn, induces IFN-γ. Alternatively, the effects of IFN-α/β on MAIDS may be mediated by the direct antiviral effects on LP-BM5 retroviruses (inhibition of virus replication) or antiproliferative effects on infected cells (slowing of viral replication in growth-arrested cells). Further studies are being undertaken to distinguish between these possibilities.

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